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Nesting in bark – the peculiar life history of the rare boreoalpine osmiine bee *Osmia* (*Melanosmia*) *nigriventris* (Hymenoptera, Megachilidae)

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Abstract

Osmia (*Melanosmia*) *nigriventris* (Zetterstedt) is a predominantly boreoalpine megachilid bee species, whose biology is poorly known due to its pronounced rarity all over Central Europe. The discovery of 19 nests in the Grisons and Valais (Switzerland) allowed for the investigation of its nesting biology and prompted the assessment of the species' host-plant spectrum, phenology and distribution. All nests were in thick bark pieces of *Larix decidua*, which lay in grassy vegetation on sun-exposed ground of open subalpine forests dominated by larch. The nests contained 1–26 brood cells constructed within one to several burrows tunneled out by the female bees with their specialized mandibles, which are distinctly stronger than those of related *O.* (*Melanosmia*) species known to nest in preexisting cavities or loose soil. The linearly arranged brood cells were separated from each other by three-layered partitions consisting of an interlayer of densely packed small bark particles sandwiched between two thin layers of chewed green leaves ("leaf pulp"). DNA metabarcoding of several nest plugs revealed that *Potentilla* (Rosaceae) and *Helianthemum* (Cistaceae) served as source of the leaf pulp. *Anthrax anthrax* (Bombyliidae) and *Sapyga similis* (Sapygidae) parasitized the brood cells of *O. nigriventris* as shown by DNA barcoding of prepupae overwintering in the host's nests. *O. nigriventris* is mesolectic and harvests pollen almost exclusively on Fabaceae (e.g. *Lotus, Hippocrepis*), Ericaceae (*Rhododendron, Vaccinium*) and Cistaceae (*Helianthemum*). Depending on the altitude, *O. nigriventris* emerges from the beginning of May to the first decade of June and thus qualifies as an early flying bee active in spring and early summer similar to the other European *O.* (*Melanosmia*) species. It likely needs two years for its development in the subalpine zone of the Alps and overwinters as prepupa in the first and probably as imago in the second winter. *O. nigriventris* has a Holarctic distribution, its disjunct Palaearctic range encompasses the boreal zone from Scandinavia eastwards to the Russian Far East, the Caucausus, the Alps as well as scattered locations at lower altitudes throughout Central Europe, where it is regarded as a glacial relict and has probably suffered strong declines during the last decades.

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Introduction

The osmiine bees (Megachilidae) are famous for their often spectacular and highly diverse nesting biology (Malyshev 1937, Cane et al. 2007, Cane 2012, Westrich 2018, Müller 2019 and references therein). The majority of species build their brood cells within preexisting cavities, such as insect borings in dead wood, hollow plant stems, rock crevices or empty snail shells. Other species glue their nests freely to a substrate or excavate them in the pith of plant stems or in the ground, occasionally also in dry dung pads. While nesting in self-excavated burrows in pithy stems or in the ground is widespread among osmiine bees and has each independently evolved in several groups (Sedivy et al. 2013), tunneling out nests in wooden substrate seems to be surprisingly rare, which strongly contrasts with the widespread use of dead wood as nesting substrate by the numerous osmiine bees that colonize borings of saproxylic insects. Self-excavation of nests in dead wood is known so far only from the Palaearctic *Osmia* (*Melanosmia*) *pilicornis* Smith, which was recently found to gnaw out its nests in branches lying on the ground (Prosi et al. 2016), and from the Nearctic *O.* (*Melanosmia*) *bucephala* Cresson, which was recorded to tunnel out burrows in a maple tree (Packard 1868). A similar behaviour was also suspected for the Holarctic *O.* (*Melanosmia*) *nigriventris* (Zetterstedt), which was observed to at least partly excavate its nests in wooden substrate (Giraud 1861, Frey-Gessner 1881, Amiet et al. 2004).

In summer 2017, a female of *Osmia leaiana* (Kirby) was observed entering a small piece of bark of larch (*Larix decidua* Mill.) lying on the ground of a subalpine larch forest near Brienz (Grisons, Switzerland). The dissection of the bark piece revealed that the female of *O. leaiana* had built several brood cells within a preexisting burrow, which was part of a larger burrow system excavated by an unknown osmiine bee species probably the year before. The large size of the burrows, which measured about 7 mm in diameter, the composition of remnants of the last year's larval provisions, which consisted of pollen of *Hippocrepis* and *Lotus* (both Fabaceae), and the presence of a dead specimen of the sapygid wasp *Sapyga similis* (Fabricius), which mainly parasitizes *Osmia* species of the subgenus *Melanosmia* (Stoeckhert 1933, Zanden 1982, Westrich 2002, 2018, Amiet 2008), all pointed to *Osmia nigriventris* as the original nest owner. DNA barcoding of a dead larva found in the old part of the bark nest later confirmed this identification (C. Praz and A. Müller, unpublished data). These findings are in line with observations made by Frey-Gessner (1881) and F. Amiet (personal communication), who each discovered a single nest of *O. nigriventris* in a small piece of thick bark of pine (*Pinus sylvestris* L.) lying on the ground, and by Stöckhert (1933), who observed two females of *O. nigriventris* nesting in the thick bark of a pine stump. These anecdotal observations suggest that the construction of nests in self-excavated burrows in bark might be the preferred nesting habit of *O. nigriventris* in Central Europe.

Osmia nigriventris is a predominantly boreoalpine megachilid bee of 10–14 mm length (Figs 26, 27). In Switzerland, it is a rare species restricted to the western and eastern Central Alps, where it inhabits open forests, windfalls and forest edges of the upper montane and subalpine zone between 1200 m a.s.l. and the timber line (SwissBeeTeam 2019). Its biology is only fragmentarily known and has never been the subject of a detailed study. In the present publication, we describe nesting site, nest architecture and nesting behaviour of *O. nigriventris* based on the recent discovery of 19 nests in the Grisons and Valais (Switzerland), identify nest building material and brood parasites by DNA analysis, apply a biomechanical model to assess the female mandibular strength and investigate the species' host-plant spectrum by microscopical analysis of female pollen loads and brood cell provisions. Furthermore, we characterize the phenology of *O. nigriventris* in Central Europe and present a distribution map of the species' entire range.

Methods

In summer 2017 and fall 2018, six sites in the Swiss Alps, where *O. nigriventris* was known to occur, were searched for its nests, i.e. Brienz/Grisons (46°40'25"N, 9°34'55"E, 1280 m a.s.l.; larch-meadow forest, Fig. 2), Schmitten/Grisons (46°41'30"N, 9°41'50"E, 1300 m a.s.l.; larch-meadow forest, Fig. 3), Curaglia/Grisons (46°39'58"N, 8°50'30"E, 1620 m a.s.l.; windfall, Fig. 5), Pfäfers/St Gallen (46°58'04"N, 9°30'00"E, 1570 m a.s.l.; windfall, Fig. 6), Calfeisen/St Gallen (46°55'27"N, 9°20'43"E, 1580 m a.s.l.; windfall) and Blatten (Lötschen)/Valais (46°26'00"N, 7°50'50"E, 1880 m a.s.l.; open larch forest, Fig. 4). At each site, pieces of larch or pine bark lying on the ground were checked for potential nests of *O. nigriventris* as was the thick bark of coniferous tree stumps or still living trees up to a height of 1.5 m. Bark pieces containing potential nests as suggested by circular holes of about 7 mm in diameter or nest plugs built from masticated green leaves (= "leaf pulp") were transported back to the lab. Nests collected in 2017 were directly dissected with a pocket knife, whereas those collected in 2018 were X-rayed first at the Animal Hospital of the University of Zurich using a Bucky Diagnost CS/TH X-Ray (Philips) combined with a Profect CS Mammo-Reader (Fujifilm), before they were opened.

To identify the source of the nest building material used by *O. nigriventris*, DNA metabarcoding of nest plugs consisting of leaf pulp was performed by the ISO 9001:2008 certified and ISO 17025:2005 accredited laboratory of Eurofins Medigenomix GmbH (Ebersberg, Germany). DNA of a sample of 5 g plant material including nine walls of six nest plugs from Blatten (Lötschen)/Valais was extracted with the Maxwell 16 FFS nucleic acid extraction kit (Promega) following the manufacturer's manual. From the extracted DNA, barcoding sequences of the nuclear marker ITS2 and the chloroplast marker

trnL were PCR amplified using target specific next-generation sequencing primers and analysed by amplicon sequencing on the Illumina MiSeq platform. The amplified sequences were sorted into sequence sets according to their similarity, each represented by a master sequence. The master sequences were identified to species or genus level by comparing them with known plant sequences made available by the NCBI database.

To compare the female mandibular strength of *O. nigriventris* with that of eight related European *Osmia* species of the subgenus *Melanosmia* with differing nesting biologies, we adapted a biomechanical model used to estimate the mandibular strength of grasshoppers (Ibanez et al. 2013) to bees (see Suppl. material 1). In contrast to the grasshopper model, the bee model does not differentiate between the incisive and the molar strength of the mandible as the bee mandible does not possess a distinct molar region, which the grasshoppers use to chew the leaf fragments they have cut with the incisive region. The bee model is described by $F = F_A \times (L_A/L_M) \times 1/R_C$, where F is the force of the mandible, F_A the force of the adductor muscle, L_A the lever of the adductor muscle, L_M the lever of the mandible and R_C the width of the cutting margin. As the force of the adductor muscle cannot be measured directly, F_A was estimated by the mandible section area expected to be correlated with the volume of the adductor muscle. The model translates into: the stronger the adductor muscle and the greater the length of L_A relative to L_M, the greater the mechanical advantage and the larger the force that can be generated at the mandible tip. Measurements were done on photographs of amputated mandibles taken with the digital microscope Keyence VHX-2000 using the free software ImageJ version 1.52a (http://imagej.nih.gov/ij). For each bee species, the force of the right mandible of three females was assessed except for *O. alticola* Benoist, *O. maritima* Friese and *O. steinmanni* Müller, for which only one or two specimens were available.

To identify brood parasites of *O. nigriventris*, DNA barcoding of 17 prepupae originating from five nests discovered near Blatten/Valais was performed. DNA was extracted with a "NucleoSpin Tissue" kit (Macherey-Nagel) following the manufacturer's protocol. The 658 base-pair-long barcoding fragment of the mitochondrial gene Cytochrome Oxidase I was amplified using the primers LepF and LepR (Hebert et al. 2004). PCR products were purified enzymatically using a mix of the enzymes Exonuclease and FastAP Thermosensitive Alkaline Phosphatase (Fermentas). Sequencing was performed in only one direction with the primer LepR by Microsynth AG. Chromatograms were edited in Geneious R6 (Kearse et al. 2012) and the exported fasta sequences were compared to existing DNA barcode libraries on BOLD (www.boldsystems.org).

To assess the pollen host spectrum of *O. nigriventris*, the scopal pollen contents of 50 females from museum and private collections originating from 41 different localities distributed across the Alps and the larval provisions in four brood cells of three nests from two different localities were microscopically analysed. The methodology for pollen removal, manufacture of pollen samples, pollen identification and data evaluation follows Müller (2018). For each brood cell, the proportions of the different pollen types were estimated to the nearest 5%.

To clarify the phenology and distribution of *O. nigriventris*, we conducted a comprehensive literature survey, gathered unpublished records from public and private entomological collections and retrieved distributional data from several public databases. For details see Acknowledgments and Suppl. material 3, which contains a complete list of all distributional data. For the phenology, only records from Central Europa (Austria, Czech Republic, France, Germany, Italy, Poland, Switzerland and Slovakia) were considered.

Results

Nesting biology

Nesting site

At three study sites – all open larch forests or larch-meadow forests (Figs 2–4) –, a total of 19 nests of *O. nigriventris* was found, 13 of which were abandoned and at least one year old, whereas six contained bee immatures upon discovery (Tab. 1). Some of the old nests were used as nesting sites by other aculeate hymenopterans in the year of discovery, such as the osmiine bee *Osmia leaiana* and the eumenine wasp *Euodynerus quadrifasciatus* (Fabricius) (Figs 22–24). All 19 nests were in bark pieces of *Larix decidua*, which lay in grassy vegetation on sun-exposed ground (Figs 1, 7, 17, 19). The bark pieces measured 13–73 cm in length, 8.5–34 cm in width and 3–8 cm in thickness (Tab. 1). Eight bark pieces contained one nest, four pieces two nests and one piece three nests. No nests were found in the thick bark of coniferous tree stumps or still living trees. The three study sites where no nests were found were all windfalls lacking thick-barked conifers, such as *Larix* or *Pinus* (Figs 5, 6).

Nest architecture

The 19 nests were all tunneled out by the female bees with no indication that preexisting burrows e.g. of cerambycid beetle larvae were used. The nesting burrows were carefully carved out and measured 6–7 mm in diameter. They were neither lined with glandular secretions nor with leaf pulp except for several small areas of few mm^2 covered with a thin layer of leaf pulp, which was probably applied to smooth out irregularities or to fill small cracks. The nest entrances, which were circular in shape (Fig. 8) and had a diameter of 6.5–7 mm, were either on the lateral side of the bark piece (n = 12; Fig. 9), on the upper side, i.e. facing upwards (n = 6; Fig. 7), or on the underside, i.e. facing to the ground (n = 1; Fig. 8, Tab. 1). In the latter case, the space between the ground and the

Figures 1–6. Habitats of *Osmia nigriventris* in the Swiss Alps. **1, 2)** Larch-meadow forest near Brienz/Grisons with nesting site - a small piece of larch bark lying on the ground (left). **3)** Larch-meadow forest near Schmitten/Grisons. **4)** Open larch forest near Blatten (Lötschen)/Valais. **5)** Windfall near Curaglia/Grisons. **6)** Windfall near Pfäfers/St Gallen.

nest entrance was relatively large, granting easy access for the nesting bee. The entrances of those seven nests, which did not start on the lateral side of the bark piece, were on the scaly exterior surface of the bark (n = 4; Fig. 7) or on the smooth interior surface (n = 3; Fig. 8).

The nests consisted either of a single linear to curved burrow (n = 9; Figs 17, 18, 21), or of a burrow system comprising 2–4 mostly linear fingers (n = 10; Figs 19, 20, 22–24, Tab. 1), which were usually excavated in roughly the same plane and often ran more or less parallel to each

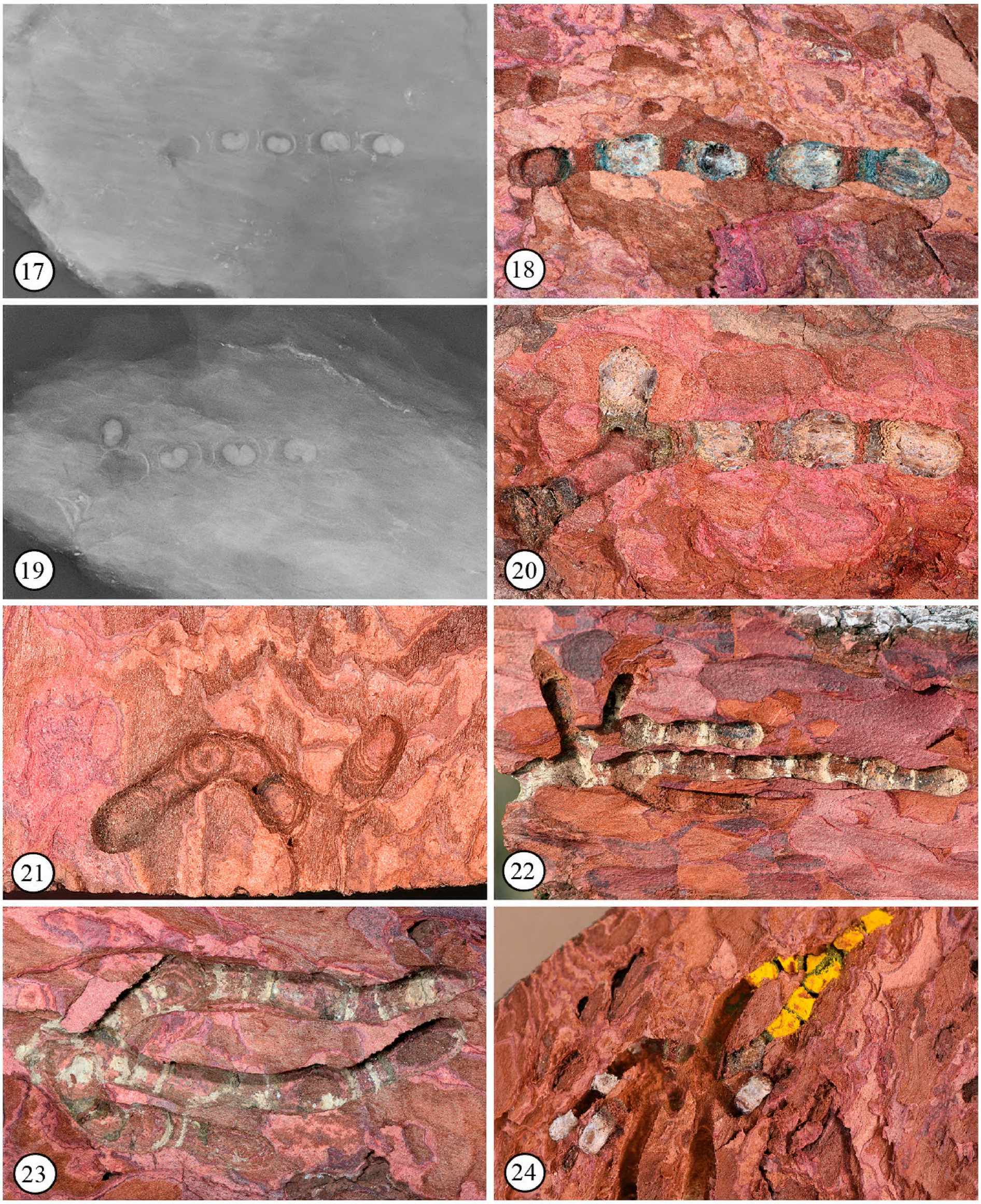
Figures 7–16. Nest architecture of *Osmia nigriventris*. **7–9)** Nest seals of nests 19, 16 and 18. **10)** Five-layered nest plug of nest 18. **11)** Single-layered nest plug of nest 17. **12)** Ring-shaped threshold. **13, 14)** Three-layered cell partitions. **15)** Brood cells with larval provisions. **16)** Brood cells with larval cocoons.

Table 1. Characteristics of 19 nests of *Osmia nigriventris* discovered in 13 bark pieces of *Larix decidua* lying on the ground at three different localities in the Swiss Alps. GR = Grisons, VS = Valais; * = nest probably built by more than one female during one or several consecutive years; b = short blind burrow without cell, suggesting that the nest was not yet finalized.

Nesting site no	Locality	Year of discovery	Maximal length × maximal width × maximal thickness of bark piece	Nest no	Nest status upon discovery	Localization of nest entrance in lying piece of bark (side of bark)	Number of brood cells	Number of burrow fingers	Number of brood cells per burrow finger	Total length of the burrow system
1	Brienz/GR	2017	29 × 13.5 × 7 cm	1	old	lateral (-)	12	5	1/1/3/3/4	27.6 cm
2	Brienz/GR	2017	31 × 28 × 5 cm	2	old	lateral (-)	10	5	1/1/2/2/4	25.5 cm
3	Brienz/GR	2017	40 × 20 × 6.5 cm	3*	old	lateral (-)	26	9+b	1/2/2/2/3/3/4/4/5	67.5 cm
				4	old	lateral (-)	7	4	1/1/2/3	15.3 cm
				5	old	lateral (-)	4	1	4	8.8 cm
4	Brienz/GR	2017	32 × 19 × 7 cm	6	old	lateral (-)	8	3	2/3/3	17.8 cm
				7	old	lateral (-)	3	1	3	6.5 cm
5	Brienz/GR	2017	13 × 8.5 × 4.5 cm	8	old	lateral (-)	2	1+b	2	6.4 cm
6	Schmitten/GR	2017	36.5 × 10.5 × 3 cm	9	old	above (exterior side)	11	5	1/2/2/2/4	29.8 cm
7	Blatten (Lötschen)/VS	2018	27 × 13.5 × 3.5 cm	10	old	lateral (-)	1	1+b	1	3.4 cm
8	Blatten (Lötschen)/VS	2018	27 × 13 × 5 cm	11	old	lateral (-)	7	?	?	?
9	Blatten (Lötschen)/VS	2018	36 × 32 × 6 cm	12	old	above (exterior side)	1	1+b	1	6.5 cm
				13	old	above (exterior side)	6	3	1/1/4	11.9 cm
10	Blatten (Lötschen)/VS	2018	54 × 20.5 × 6.5 cm	14	occupied	above (interior side)	2	1	2	6.7 cm
				15	occupied	above (interior side)	2	1	2	5.3 cm
11	Blatten (Lötschen)/VS	2018	51 × 29 × 3.5 cm	16	occupied	below (interior side)	4	1	4	9.2 cm
12	Blatten (Lötschen)/VS	2018	26 × 16 × 8 cm	17	occupied	lateral (-)	3	1	3	6.0 cm
				18	occupied	lateral (-)	4	2	1/3	8.3 cm
13	Blatten (Lötschen)/VS	2018	73 × 34 × 6 cm	19	occupied	above (exterior side)	4	2	1/3	9.5 cm

other. Except for the 9–14 mm long and almost vertical entrance burrow of those nests that did not start on the lateral side of the bark piece, the nesting burrows ran more or less parallel to the upper and under surface of the bark. The total length of the excavated burrows per nest was correlated with the number of brood cells and ranged from 3.4–29.8 cm (Tab. 1). The nests contained 1–12 brood cells, which were either constructed singly in short burrows (Figs 19, 20) or in a linear series of 2–4 in longer burrows (Figs 15–24, Tab. 1). The brood cells had a length of 10–14 mm and were widest in the middle (6.5–9 mm) and slightly constricted at both ends (6–7 mm). Nest 3 was an exception in that its burrow system had a total length of 67.5 cm and comprised 26 brood cells distributed in nine fingers, which were tunneled out in three different planes within the bark and contained up to five linearly arranged cells (Tab. 1). In contrast to all the other nests, which were most probably constructed by a single female only, the high number of brood cells suggests that nest 3 was likely built by several females, either in the same or in consecutive years.

Three types of partitions were recorded in the nests of *O. nigriventris*: i) partitions that separate the brood cells from each other ("inner cell partitions"); ii) partitions that seal the outermost brood cell towards the nest entrance or a finger towards its mouth ("outer cell partitions"), and iii) partitions that close the nest at the entrance ("nest plug"). The inner cell partitions, which ranged in length from 4–12 mm, were invariably three-layered (n = 24; Figs 13–16), consisting of a central layer of densely packed small bark particles (2–9 mm long) sandwiched between two layers of leaf pulp (marginally 1–2 mm thick). As revealed by two thresholds of leaf pulp at the entrance of two still empty brood cells (Fig. 12), the females mark the position of the later cell partition before they begin to provision a brood cell; these two thresholds were ring-shaped, which seems to be typical also for other *Osmia* species of the subgenus *Melanosmia*, such as *O. parietina* (A. Müller, unpublished data). The construction of the outer cell partitions, which ranged in length from 2–10 mm, was slightly more variable than that of the inner cell partitions: most of them were three-layered as the inner cell partitions and had similar dimensions (n = 9), whereas some partitions at the mouth of a finger were either five-layered with three layers of leaf pulp (marginally 1–2 mm thick) enclosing two layers of small bark particles (1–2 mm long; n = 3) or consisted of a single leaf pulp layer only (marginally 2 mm thick; n = 3). The construction of the nest plugs, which were usually flush with the nest opening, considerably varied between the different nests (Figs 10, 11): the plugs ranged from 3–11 mm in length and consisted either of a single layer of leaf pulp (marginally 3–5 mm thick; n = 3), of two layers of leaf pulp (marginally 1.5–3.75 mm thick) enclosing a 1 mm long layer of small bark particles (n = 1), of three layers of leaf pulp (marginally 2–3 mm thick) enclosing two slightly less than 1 mm long layers of bark particles (n = 1), or of five layers of leaf pulp (marginally 1.5–2



Figures 17–24. Nests of *Osmia nigriventris*. **17, 18)** Nest 16 X-rayed (left) and dissected (right). **19, 20)** Nest 18 X-rayed (left) and dissected (right). **21)** Old nest 8 with short blind burrow on the right. **22–23)** Old nests 2 and 6 later used by *Euodynerus quadrifasciatus* as nesting site. **24)** Old nest 1 with brood cells of *Osmia leaiana* in the upper half and remnants of the old nest of *O. nigriventris* in the lower half.

mm thick) enclosing four 1–3 mm long layers of bark particles (n = 1). The length of the vestibule, i.e. the distance between the interior side of the nest plug and the outermost layer of the outer cell partition, ranged from 0.5–2.8 cm.

DNA metabarcoding revealed that the plugs of nests 14–19 were constructed from masticated leaves of *Potentilla* spec. (Rosaceae) and *Helianthemum nummularium* (Cistaceae) (Tab. 2). In addition, a low proportion of reads corresponded to *Lotus corniculatus* (Fabaceae).

Table 2. Origin of the masticated green leaves used by *Osmia nigriventris* to build the nest plugs based on DNA metabarcoding of a sample of nine walls from nests 14–19 (see Tab. 1).

Plant taxon	% sequence reads
Potentilla spec. (Rosaceae)	76.3
Helianthemum nummularium (Cistaceae)	22.5
Lotus corniculatus (Fabaceae)	1.2

Although the percentage of sequence reads obtained by next-generation sequencing is only a rough indication for the relative amount of a plant taxon in the extracted DNA, this low proportion might be due to a contamination e.g. with pollen of *Lotus*, which is the most important pollen host of *O. nigriventris* in the Alps (see below).

Female mandibular strength

The assessment of the mandibular strength revealed a low intraspecific variability indicating that the shape and the force of the female mandibles are species-specific traits (Suppl. material 2). Among the nine *O.* (*Melanosmia*) species examined, *O. nigriventris* and *O. pilicornis*, which both excavate their nests in wooden substrate, possess by far the strongest mandibles (Fig. 25, Suppl. material 2). In contrast, the mandibles of those species, which either nest in preexisting cavities (*O. inermis, O. parietina*, *O. uncinata*) or in loose soil and litter (*O. alticola, O. maritima, O. xanthomelana*), are distinctly weaker. Compared with these species, the mandibles of *O. nigriventris* are relatively shorter, have less diverging margins and are apically only slightly curved inwards resulting in a compact and chisel-like shape that appears to be well suited to tunnel out burrows in bark or dead wood (Figs 28, 29).

Brood parasites

The obtained sequences of the barcoding gene Cytochrome Oxidase I were all clean and readable. Comparison with BOLD databases indicate that 13 out of the 17 overwintering prepupae belong to *O. nigriventris*, whereas two prepupae each from the nests 15 and 19 were

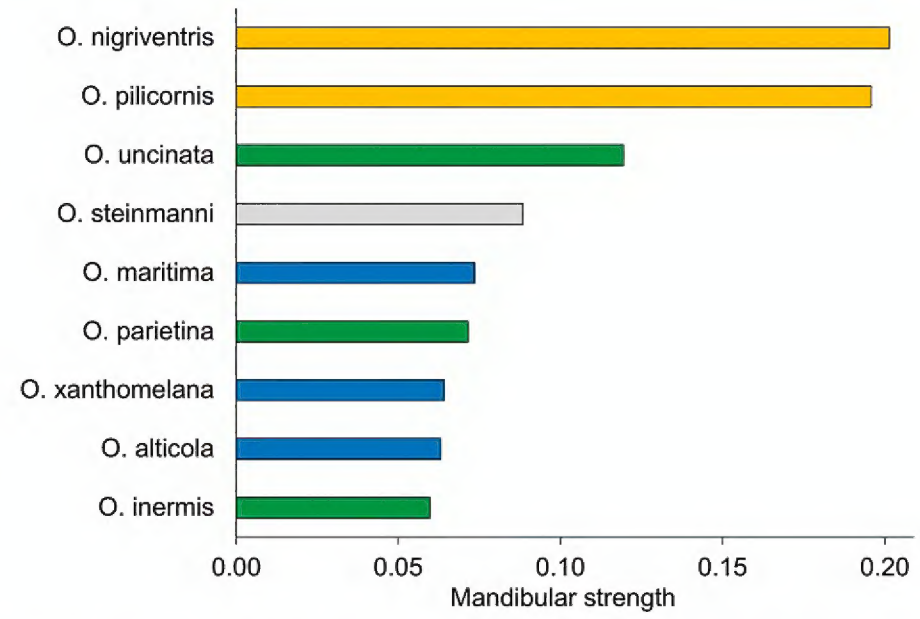


Figure 25. Mandibular strength of nine European *Osmia* species of the subgenus *Melanosmia*. For each species, the force of the right mandible of three females was averaged except for *O. alticola*, *O. maritima* and *O. steinmanni*, for which only one or two specimens were available. Orange = nesting in self-excavated burrows in wooden substrate, green = nesting in preexisting cavities, blue = nesting in loose soil or litter, grey = nesting unknown (after Müller 2019 and references therein).

brood parasites, i.e. three specimens of the bombyliid fly *Anthrax anthrax* (Schrank) and one specimen of the sapygid wasp *Sapyga similis* (Fabricius). From the latter species, a dead imago was also found in a brood cell of nest 1. The percentage of match between the barcoding sequences of the 17 prepupae and the BOLD reference sequences exceeded 99% in all cases.

Pollen hosts

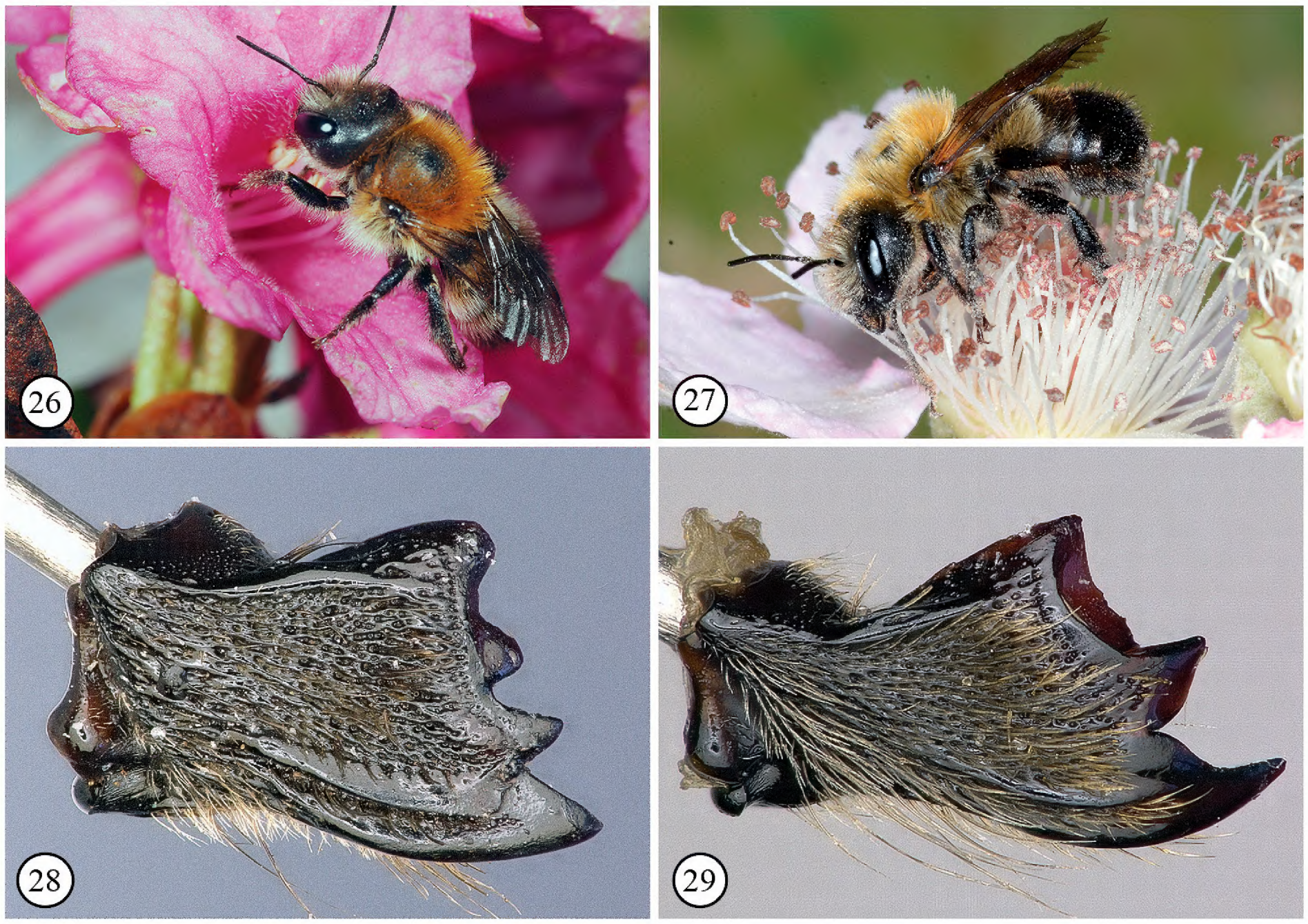
Osmia nigriventris collected the pollen almost exclusively from flowers of Fabaceae, Ericaceae and Cistaceae (Tab. 3, Figs 26, 30). Pollen of these three plant families constituted 96.6% of the total pollen grain volume. Among the Fabaceae, *Lotus* was by far the most important pollen host. Its pollen was represented by 55.8% of the total pollen grain volume and was recorded in 42 out of 50 pollen loads. Among the Ericaceae, both *Rhododendron* and *Vaccinium* were exploited. Their pollen contributed 18.1% and 8.5%, respectively, to the total

Table 3. Pollen composition of female pollen loads of *Osmia nigriventris*. n = total number of pollen loads, N = number of pollen loads from different localities. Countries: CH = Switzerland, F = France. Plant families: BOR = Boraginaceae, CIS = Cistaceae, FAB = Fabaceae, ERI = Ericaceae. Definitions of bee host range after Müller and Kuhlmann (2008).

n	N	Origin (and number) of pollen loads	% pollen grain volume (number of loads)	Preferred host	% pollen grain volume of preferred host	% pure loads of preferred host	% loads with preferred host	Host range
50	41	CH (48), F (2)	FAB (*Lotus*) 55.8% (42), FAB (*Hippocrepis*) 5.4% (7), FAB (*Anthyllis*) 1.6% (3), FAB (*Trifolium*) 1.2% (3), FAB (*Vicia/Lathyrus*) 0.5% (2), ERI (*Rhododendron*) 18.1% (10), ERI (*Vaccinium*) 8.5% (7), CIS (*Helianthemum*) 5.6% (5), GEN (*Gentiana*) 2.1% (1), BOR (*Echium*) 0.9% (1), unknown 0.3% (2)	Fabaceae, Ericaceae and *Helianthemum* (Cistaceae)	96.7%	96.0%	100%	mesolectic on Fabaceae, Ericaceae and *Helianthemum* (Cistaceae)

Table 4. Pollen composition of larval provisions in four brood cells of *Osmia nigriventris* from three nests.

Locality	Nest no	Brood cell no	Fabaceae: *Lotus*	Fabaceae: *Hippocrepis*	Ericaceae
Brienz/GR	nest 3	brood cell	95%	5%	
Blatten (Lötschen)/VS	nest 13	brood cell	70%	5%	25%
Blatten (Lötschen)/VS	nest 14	brood cell 1	50%	5%	45%
		brood cell 2	80%		20%
			73.75%	**3.75%**	**22.5%**



Figures 26–29. 26, 27) Females of *Osmia nigriventris* collecting pollen on *Rhododendron ferrugineum* (left, photo H. Wiesbauer) and sucking nectar on *Rubus* (right, photo S. Kühne). **28)** Female mandible of *Osmia nigriventris*. **29)** Female mandible of *Osmia inermis*.

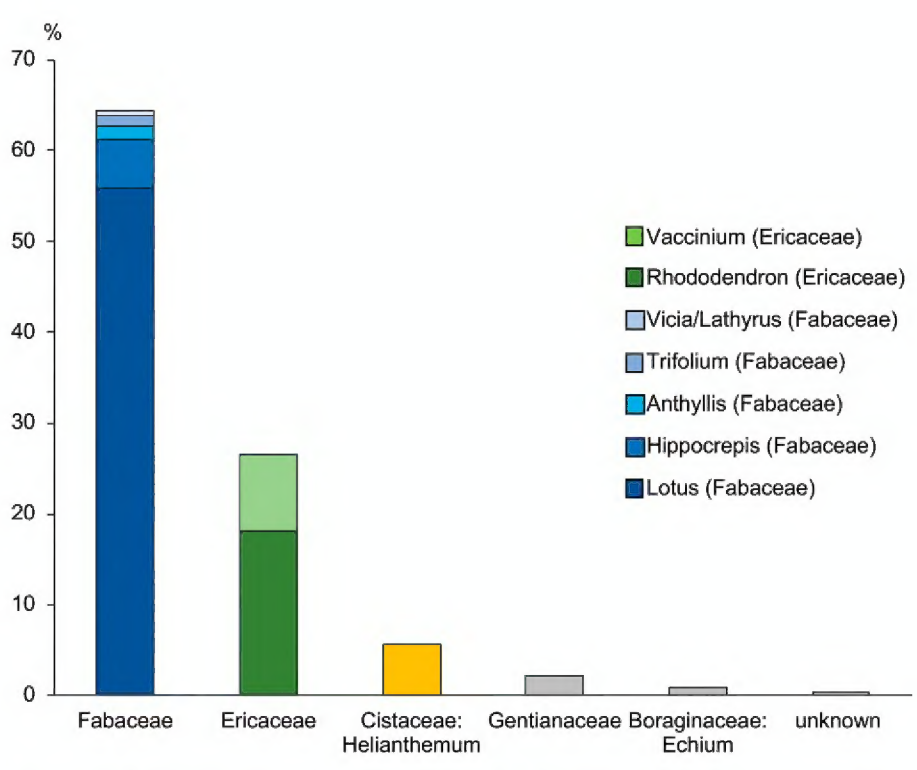


Figure 30. Pollen host spectrum of *Osmia nigriventris* based on the microscopical analysis of 50 scopal loads from 41 different localities in the Swiss and French Alps.

pollen grain volume and was found in 10 and 7 loads, respectively, rendering these two genera the second and third most important host taxon of *O. nigriventris*. *Helianthemum* (Cistaceae) was the fourth most important host taxon. Its pollen constituted 5.6% of the total pollen grain volume and was recorded in 5 loads.

The larval provisions of four brood cells originating from three nests contained only pollen of Fabaceae and Ericaceae (Tab. 4). One provision exclusively consisted of Fabaceae pollen, whereas the other three provisions were mixtures of the pollen of both families. *Lotus* was the most important pollen host in each of the four provisions.

Phenology and overwintering stage

In Central Europe, the imaginal period of *O. nigriventris* extends from May to August (Fig. 31). At the lowest extra-alpine occurrences at elevations below 900 m a.s.l., the flight activity might start as early as the beginning of

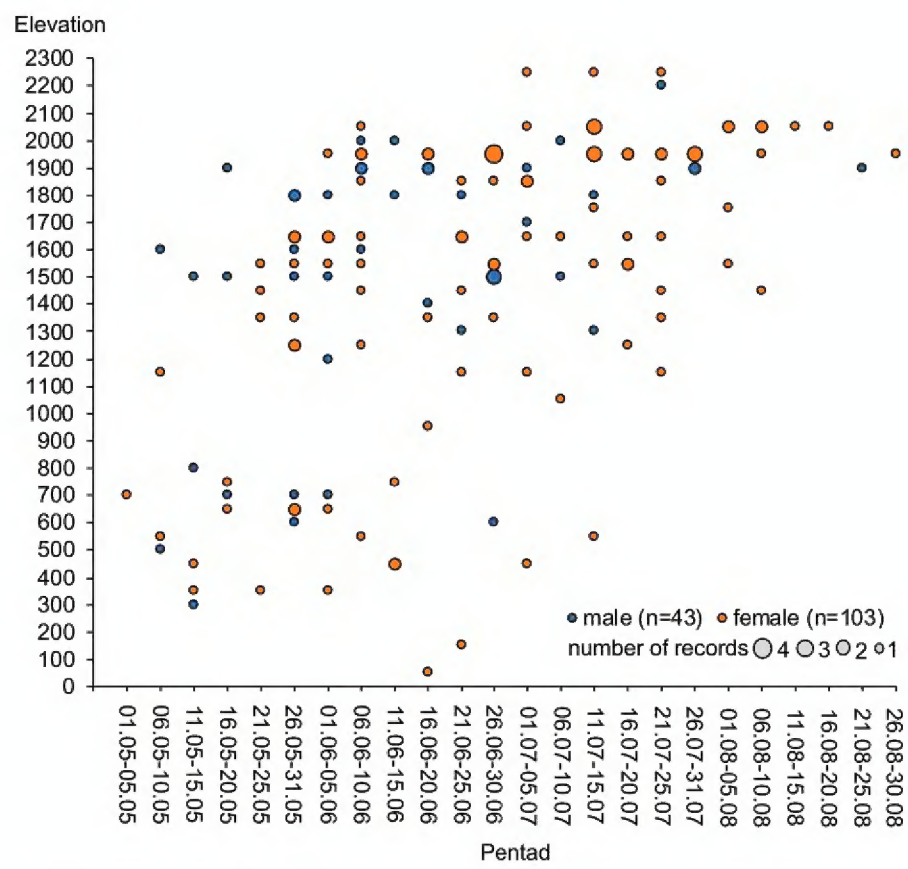


Figure 31. Phenology of *Osmia nigriventris* in Central Europe. For each pentade, the number of female and male records per elevation is given. For a given locality and date, only one record per sex was considered.

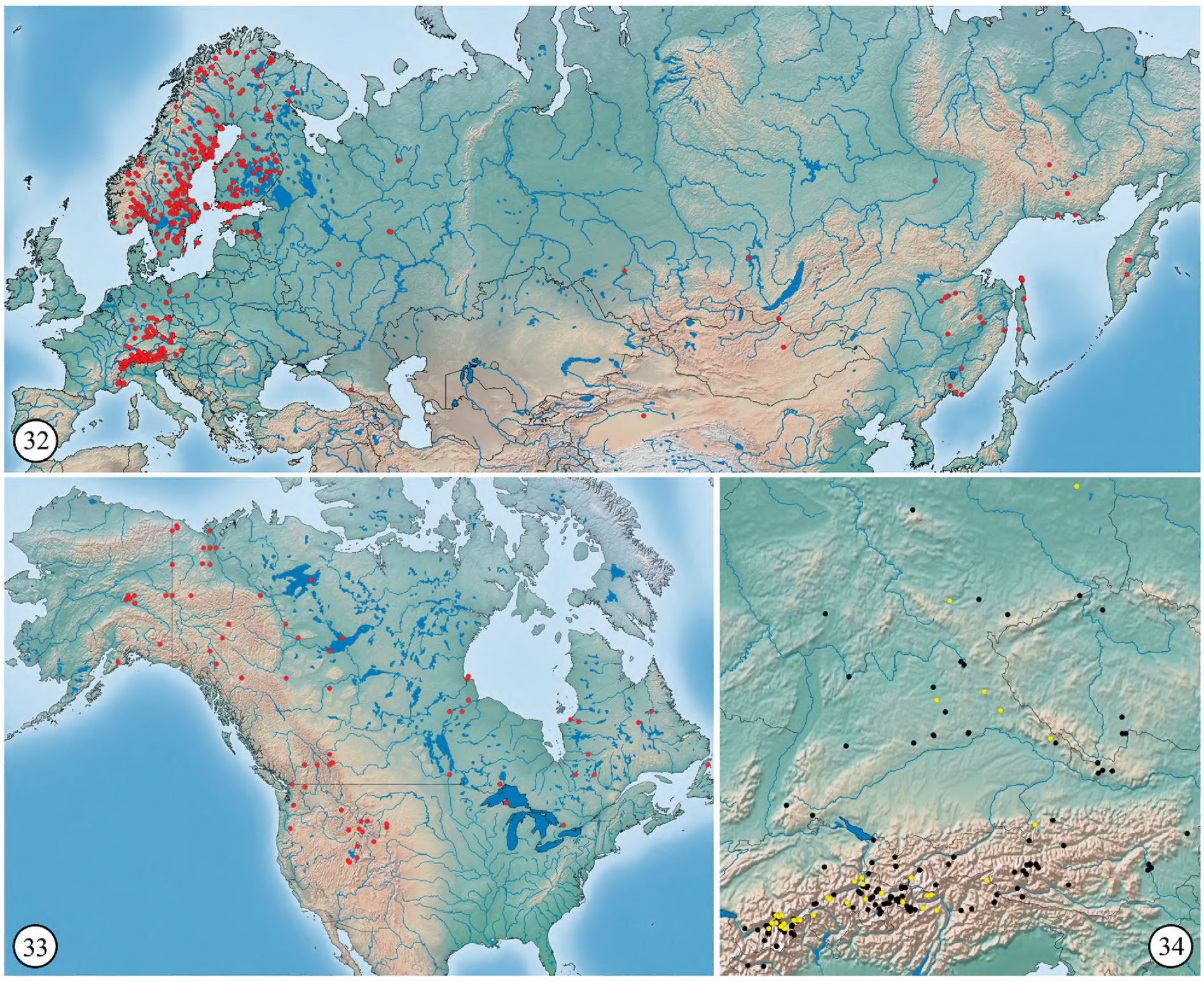
May and does not extend beyond the first decade of June, whereas near the timberline in the subalpine zone of the Alps the imagines do not emerge before the end of May and fly till the end of August.

X-raying of the nests 14–19 performed in November 2018 revealed that 13 out of the 19 brood cells contained prepupae of *O. nigriventris* spun in a cocoon (Figs 16–20), suggesting that *O. nigriventris* passes at least its first winter in the prepupal stage (see Discussion). In two cells, the eggs or the freshly eclosed larvae had died leaving behind the entire larval provisions (Fig. 15), and the remaining four cells contained prepupae of *Anthrax anthrax* and *Sapyga similis* (see above).

In one brood cell each of three abandoned nests, the adult bee had left its cocoon in spite of the fact that the cell partitions were still intact, suggesting that the imagines occasionally leave their natal nest through a new burrow gnawed across the bark rather than through the nest entrance.

Distribution

O. nigriventris has a vast Holarctic distribution (Figs 32–34, Suppl. material 3). In the Nearctic, *O. nigriventris* oc-



Figures 32–34. Distribution of *Osmia nigriventris* in **32)** the Palaearctic, **33)** the Nearctic and **34)** Central Europe. For Central Europe, records before 2000 are given in black, those from 2000 to the present in yellow. See Suppl. material 2 for a complete list of all distributional data. Made with Natural Earth (www.naturalearthdata.com).

curs in the western and northern United States (Colorado, Wyoming, Idaho, Montana, Oregon, Washington, Alaska, Minnesota, Michigan) and Canada (Alberta, British Columbia, Yukon, Northwest Territories, Manitoba, Ontario, Quebec, Newfoundland and Labrador) within a belt that extends from 40° to 69° northern latitude (see also Rightmyer et al. 2010 and Ascher and Pickering 2019). In the Palaearctic, its range is split into three main parts: i) Alps (France, Switzerland, Italy, Germany, Austria) from 1200 m a.s.l. up to the timberline at 2300 m a.s.l., ii) Caucasus and iii) the boreal zone from Scandinavia (Norway, Sweden, Finland), the Baltic (Lithuania, Latvia, Estonia) and European Russia eastwards over Siberia, northwestern China and Mongolia to northeasternmost China and the Russian Far East between 41° and 70° northern latitude. Widely scattered outposts of its distribution exist in the lowlands of northern Germany and Poland as well as in some low mountain ranges in Germany, the Czech Republic and Slovakia, where the species is regarded as a glacial relict (Figs 32, 34). Among the 53 records of *O. nigriventris* north of the Alpine arc, which range in altitude from 55 m to 730 m a.s.l., 43 (81.1%) date from before 1995, suggesting that numerous former Central European occurrences outside the Alps no longer exist.

Discussion

Nesting biology

At three different localities in the Grisons and the Valais, numerous nests of *Osmia nigriventris* were discovered, which were all constructed in thick bark pieces of *Larix decidua* lying on sun-exposed ground of open subalpine forests. Nests of *O. nigriventris* were also detected by Frey-Gessner (1881) and F. Amiet (personal communication) in pieces of *Pinus sylvestris* bark at the Alp Ponchet above Chandolin/Valais and near Samedan/Grisons, respectively, and by Stöckhert (1933) in the thick bark of a *Pinus* stump in Franken (Germany). Furthermore, a female of *O. nigriventris* was observed to fly off from its putative nest in a bark piece lying at the base of a *Pinus sylvestris* stump in Brandenburg/Germany (Saure 2017, personal communication). Lastly, L. Gyllenhall, a Swedish entomologist living from 1752 to 1840, found *O. nigriventris* nesting in the bark of a pine in Vastergötland (Sweden), which later prompted Gerstaecker (1869) to give this species the name *O. corticalis* (cortex = bark), now regarded as a junior synonym of *O. nigriventris* (Nilsson, 2009). These records of bark nests at five different localities in the Swiss Alps and at one locality each in central Germany, northern Germany and southern Sweden indicate that the construction of nests in the bark of thick-barked conifers is a typical trait of *O. nigriventris*. As numerous conifers including *Larix* and *Pinus* seal injuries of the bark with resin (Krokene 2015 and references therein), *O. nigriventris* is expected to nest only in bark which adheres to dead stumps or is detached from the stems and lies on the ground. However, *O. nigriventris* is not strictly specialized to bark as nesting substrate, as is revealed by its occurrence at localities, where no thick-barked conifers occur, such as the three windfalls investigated in the present study. Here, the species is supposed to excavate its nests in dead wood of *Picea abies* stumps. In fact, Giraud (1861) observed several females of *O. nigriventris* nesting in self-excavated burrows in a thick board of *Abies alba*, which formed part of a pasture fence. This shows that while *O. nigriventris* might have a pronounced preference for nesting in bark, it is capable of constructing its nests also in dead wood.

Among the osmiine bees, bark appears to be only exceptionally used as nesting substrate. Apart from *O. nigriventris*, reports for nesting in bark exist only for the Palaearctic species *O. uncinata* Gerstaecker and the three Nearctic species *O. atriventris* Cresson, *O. densa* Cresson and *O. cahuilla* Cooper, all belonging to the subgenus *Melanosmia*. The first three species differ from *O. nigriventris* in that they colonized already existing beetle burrows in bark and also use preexisting linear cavities in other substrates, such as dead wood or stems (Graenicher 1906, Stoeckhert 1933, Linsley and MacSwain 1951, Cane et al. 2007, Else and Edwards 2018). This suggests that the selection of bark for nesting was rather accidental in these three species. In *O. cahuilla*, 21 individuals emerged from a single nest within the bark of a pine stump (Cooper 1993). As *O. cahuilla* is hypothesized to be related to the Palaearctic *O. pilicornis* (Cooper 1993), which nests in self-excavated burrows in dead wood (Prosi et al. 2016), the bark nest might possibly have been gnawed out by the nesting female as in *O. nigriventris*.

The inner cell partitions, which consist of an interlayer of bark particles sandwiched between two thin layers of leaf pulp, are diagnostic for the nests of *O. nigriventris*. Doubtlessly, the interlayer is constructed by amassing bark particles that accumulated within the burrow during the process of nest excavation. Among the osmiine bees, which usually separate linearly arranged brood cells by one-layered walls, such three-layered cell partitions are otherwise known only from the Palaearctic *Hoplitis tuberculata* Nylander and its North American relatives (Müller 2015). In these *Hoplitis* species, the interlayer consists of pebbles, wood chips, earth crumbs, fragments of conifer needles or particles of pith. These strong cell partitions are hypothesized to impede mobile larvae of predators, which already infested a brood cell before the nest was closed, from invading adjacent cells, such as larvae of *Trichodes* beetles (Cleridae). The same function might also apply to the massive cell partitions of *O. nigriventris*.

The mandibles of *O. nigriventris* and *O. pilicornis* turned out to be the strongest among the European *O.* (*Melanosmia*) species. This finding corresponds well to the habit of both species to tunnel out their nests in hard wooden substrate and supports the view that both species are specialized excavators and do not colonize preexisting cavities for nesting, such as insect borings in dead wood. Such a specialization also explains why the repeated attempts to establish these two species in trap nests composed of drilled borings in dead wood all have failed (A. Müller and R. Prosi, unpublished data).

Anthrax anthrax (Bombyliidae) and *Sapyga similis* (Sapygidae) were recorded as brood parasites of *O. nigriventris* in the present study. Most species of *Anthrax* are ectoparasitoids of above-ground nesting aculeate Hymenoptera attacking the full-grown larvae of their hosts (Yeates 1997). *A. anthrax* was found to parasitize the crabronid wasp *Pemphredon lugens* Dahlbom, the megachilid bees *Megachile nigriventris* Schenck, *Hoplitis adunca* (Panzer), *Osmia bicornis* (Linnaeus) and *O. cornuta* (Latreille) as well as the apid bee *Anthophora retusa* (Linnaeus), revealing a wide host range (Brechtel 1986, Litt 1996, Krunić et al. 2005, Peeters et al. 2012, Westrich 2018). In contrast to *A. anthrax*, *S. similis* was already known to be an antagonist of *O. nigriventris* (Stoeckhert 1933, Amiet 2008). It develops in the nests of other *O.* (*Melanosmia*) species, such as *O. inermis* (Zetterstedt), *O. parietina* Curtis, *O. uncinata* and probably also *O. maritima* Friese and *O. xanthomelana* (Kirby) (Stoeckhert 1933, Zanden 1982, Westrich 2002, 2018), suggesting a preference for *Osmia* species of the subgenus *Melanosmia* as hosts. However, *S. similis* was also reared from nests of *Chelostoma grande* (Nylander) (Westrich 2002) and might thus be expected to parasitize further osmiine bee species occurring in the Alps and the Jura Mountains, where *S. similis* has its main distribution in Switzerland (Amiet 2008). *Chrysura hirsuta* (Gerstaecker) (Chrysididae) is most probably a further brood parasite of *O. nigriventris* (Berland and Bernard 1938, Trautmann 1927, Stoeckhert 1933, Paukkunen et al. 2014), although no proof for its development within the nests of *O. nigriventris* exists so far. As in *S. similis*, *Osmia* species of the subgenus *Melanosmia* appear to be the main hosts of *C. hirsuta* (Prosi et al. 2016, Westrich 2018).

Pollen hosts

Microscopic analysis of both female pollen loads and larval provisions revealed that *Osmia nigriventris* is mesolectic, restricting pollen harvesting almost exclusively to flowers of Fabaceae, Ericaceae and to a lesser extent *Helianthemum* (Cistaceae). Known pollen host species are *Lotus corniculatus* L. and *Hippocrepis comosa* L. among the Fabaceae, *Rhododendron ferrugineum* L., *R. hirsutum* L., *Vaccinium myrtillus* L. and *V. vitis-idaea* L. among the Ericaceae and *Helianthemum nummularium* (L.) Mill. among the Cistaceae (Frey-Gessner 1899–1907, Stoeckhert 1933, Westrich 2018, Dylewska 1993, this study). In contrast to the literature, no preference for *Vaccinium* over Fabaceae was evident in the present study as assumed by Stoeckhert (1933) and Westrich (2018), and pollen of *Potentilla* (Rosaceae), which is listed as an additional pollen source by Westrich (2018), was never recorded in any pollen load.

The pollen host spectrum of *O. nigriventris* is similar to that of other predominantly alpine *Osmia* (*Melanosmia*) species, for which pollen of Fabaceae also plays a crucial role for larval development (Müller 2018). Compared with these *O.* (*Melanosmia*) species, which comprise narrow Fabaceae specialists collecting pollen only on two closely related genera as well as generalists harvesting pollen on up to eleven different plant families, *O. nigriventris* occupies an intermediate position with respect to its degree of host plant specialization. The combined exploitation of Fabaceae and Ericaceae found to be typical for *O. nigriventris* in the Alps has also been observed in other Palaearctic *O.* (*Melanosmia*) species inhabiting Ericaceae-rich alpine and boreal habitats, such as *O. disjuncta* Tkalců, *O. inermis* (Zetterstedt) and *O. steinmanni* Müller (Hicks 2009, Müller 2018, 2019) and might possibly also apply to *O. laticeps* Thomson currently considered to be oligolectic on *Vaccinium* (Nilsson 2009).

Phenology and overwintering stage

Similar to the other European *Osmia* species of the subgenus *Melanosmia*, which are early flying bees active in spring and early summer (Westrich 2018, SwissBeeTeam 2019), *O. nigriventris* already emerges in phenological spring, i.e. between the beginning of May at low altitudes and the first decade of June at high altitudes. As the nests of *O. nigriventris* from Blatten/Valais (1880 m a.s.l.) contained prepupae upon examination in winter 2018, it seems improbable that the time span between the end of winter and the onset of the early flight period is sufficiently long to allow for the entire metamorphosis to the imaginal stage. We therefore hypothesize that *O. nigriventris* needs two years for its development in the subalpine zone of the Alps and that it overwinters here as prepupa in the first and as imago in the second winter. This hypothesis is supported by observations made on other *O.* (*Melanosmia*) species: *O. inermis* needs at least two years for its development on the German Swabian Alb and in Scotland, where the first winter is passed as prepupa and the second as diapausing adult (Else and Edwards 1996; Westrich 2002), and *O. parietina* was found to emerge not until two years after egg deposition in the Valais at 1430–1800 m a.s.l. (Westrich 2002).

Distribution

O. nigriventris belongs to the few bee species that have an autochthonous Holarctic distribution (Ebmer 2011). Although the species is not threatened at the European scale due to its wide distribution and large population size in Scandinavia (Nieto et al. 2014), it is scarce and locally distributed throughout the Alps and appears to have disappeared from many of its former Central European extra-alpine glacial refugia at low altitudes. Though this decline at low altitudes might have been caused by habitat deterioriation, it might also be a first indication that climate warming is increasingly negatively affecting population survival of this cold-adapted species, which raises concern about the species' future in the upper montane and lower subalpine zone of the Alps.

Conclusions

The nesting biology of *O. nigriventris* is exceptional among the osmiine bees in that the females excavate their nests in wooden substrate, preferentially in thick bark of *Larix* and *Pinus*, which either lies on the ground or adheres to dead tree stumps. The finding that five out of 13 bark pieces used as nesting sites contained two or more nests and that at least one nest was most probably owned by several females suggests that suitable bark might be a limiting resource for *O. nigriventris* in the Alps. In fact, the search for nests of *O. nigriventris* in the course of the present study revealed that bark pieces of thick-barked conifers lying on the ground are rare by nature and - moreover - are often removed by the farmers from the floor of grazed forests, such as larch-meadow forests. Given the rarity of *O. nigriventris* throughout the Alpine arc and its supposed decline at low and mid altitude due to climate warming, we urge land owners, foresters, farmers and local authorities to leave sufficient amounts of thick bark on the ground of open subalpine larch and pine forests.

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Supplementary material 1

Biomechanical model used to compare the mandibular strength among *Osmia* species of the subgenus *Melanosmia*

Authors: Andreas Müller, Rainer Prosi, Christophe Praz, Henning Richter

Data type: PDF file

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Supplementary material 2

Mandibular strength of nine European *Osmia* species of the subgenus *Melanosmia*

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Data type: PDF file

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Supplementary material 3

List of distributional data of *Osmia nigriventris*

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Data type: XLS file

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